1969

Reproductive Seasonality and Related Behavior in the Marsh Periwinkle, Littorina irrorata Say (Gastropoda, Prosobranchiata)

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REPRODUCTIVE SEASONALITY AND RELATED BEHAVIOR IN THE MARSH PERIWINKLE, LITTORINA IRRORATA SAY (GASTROPODA: PROSOBRANCHIATA)

A Thesis

Presented to

The Faculty of the School of Marine Science

The College of William and Mary in Virginia

In Partial Fulfillment

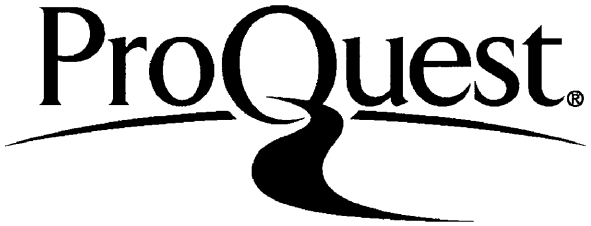
Of the Requirements for the Degree of

Master of Arts

By

Daniel G. Gibson III

1969



APPROVAL SHEET

This thesis is submitted in partial fulfillment of

the requirements for the degree of

Master of Arts

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ACKNOWLEDGMENTS

I wish to express my thanks to the National Science Foundation, which supported part of this work. Thanks are also due to Drs. Marvin Wass, Langley Wood, and Jay D. Andrews, for their critical reading of the manuscript. Miss Patsy Ann Smith and Dr. Jeanette Whipple Struhsaker made their unpublished manuscripts available to me, and allowed me to mention their results. Dr. Struhsaker's helpful suggestions were also greatly appreciated. I wish to thank Dr. Frank Perkins for his help in interpreting the prepared slides.

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ABSTRACT

Reproductive activity of a population of Littorina irrorata Say was followed from March 1967 through August 1968. Both macroscopic and microscopic methods of determining gonad maturity were employed. Recovery of encapsulated zygotes from the plankton and in the laboratory was attempted. Copulatory and migratory behavior were observed in the field.

Breeding season was roughly from May through September. Earlier maturation of gonads in 1968 was linked with warmer temperatures in May, but resorption in September could not be attributed solely to falling temperatures. Planktonic, encapsulated zygotes were released in the field throughout the lunar cycle, but only on high tides. Attempts to obtain zygotes in the laboratory were most successful at the time of spring tides. Abundance of capsules in the plankton differed markedly between the two years of observation. Copulatory position was identical with that in other Littorina; copulation occurred most often on ebb tides, on horizontal surfaces.

REPRODUCTIVE SEASONALITY AND RELATED BEHAVIOR IN THE MARSH PERIWINKLE, *LITTORINA IRRORATA* SAY (GASTROPODA: PROSOBRANCHIATA)

INTRODUCTION

Representatives of the prosobranch genus Littorina are found on rocky shores throughout the world (Thiele, 1931). The life habits and reproduction of many species have been thoroughly studied. The littorines are dioeceous, and fertilization is internal. The male possesses, on the right side of the body, a penis which becomes tumid prior to copulation and is then inserted into a genital pore at the back of the female mantle cavity (Linke, 1933a; Gibson, 1964). The penis atrophies between breeding seasons in some species, but not in others (Linke, 1933b). Testes or ovaries develop in the visceral mass of the animal prior to the breeding season; the amount of resorption after breeding varies interspecifically (Linke, 1933b). A testicular duct is visible along the columellar side of the abdomen in males when filled with sperm; in females the capsule gland is enlarged during the breeding season (Fretter and Graham, 1962). These structures permit identification of breeding individuals and of sex.

Seasonality in breeding is pronounced in most temperate species, whereas it is not apparent in tropical littorines (Struhsaker, 1966). Gonad development is associated with rising environmental temperatures in some species, and with falling temperatures in others (Rohlack, 1959; Lysaght, 1941). Temporally exclusive breeding seasons in sympatric species have been reported from Israel (Palant and Fishelson, 1968); overlapping breeding seasons and interspecific

copulation have been described in North Sea species (Linke, 1933a, b). Both starvation and summer-level temperatures were found effective in causing resorption of testes and penes in males of Littorina littorea (Linnaeus), which breeds in the spring (Linke, 1934). In spite of increasing photoperiod, experimentally lowered temperatures prolonged reproductive condition in L. littorea that were already sexually mature, and induced early maturation in previously spent animals (Rohlack, 1959). Littorina littorea require four years to mature sexually in the Arctic temperatures of the White Sea (Rubinchick, 1961), but only two years in Wales (Williams, 1964). An estrogen-like hormone has been isolated from fertile L. littorea females, but no male hormone has been demonstrated (Rohlack, 1959).

Type of spawn differs greatly among littorines. Although eggs are always fertilized internally, the stage of development at the time of release, and the mode of release, both vary interspecifically. Zygotes in pelagic capsules are released in a majority of species. The normal number of zygotes per oötheca is usually one, but fourteen per capsule occur normally in at least one species (Kojima, 1958a). In other species, capsules are attached to substrates, where the embryos develop to juvenile snails before hatching. Brooding of juveniles to crawling stages within the mother has also been reported (Linke, 1933b; Lebour, 1935). In at least one species, free veligers are released alternately with pelagic zygote capsules on successive spring highs (Lenderking, 1954).

Lunar periodicity in release of pelagic capsules, i.e., release on spring highs, occurs in many species (Lysaght, 1941; Lenderking, 1954; Struhsaker, 1966). Release of capsules in the laboratory

concomitant with high tide in the field has been demonstrated (Lenderking, 1954; Struhsaker, 1966). The young of some species have been reared in the laboratory through metamorphosis (Struhsaker, 1966; Struhsaker and Costlow, 1968).

Most Littorina are found on rocky shores, where as many as four species occur at different intertidal levels on the same coast (Lebour, 1937; Fretter and Graham, 1962). Two species which do not live on open, rocky coasts are the mangrove swamp periwinkle, Littorina angulifera Lamarck, and the salt marsh periwinkle, Littorina irrorata Say. Each is the only representative of the genus in its particular habitat. Littorina angulifera is a subtropical species found in the mangrove swamps of Florida and the Caribbean islands (Baquaert, 1943). Its reproductive habits have been thoroughly studied by Lenderking (1954).

Littorina irrorata is abundant in salt marshes from Long Island to Florida on the Atlantic coast, and from Florida to Texas in the Gulf of Mexico (Bequaert, 1943). These small snails are found in a wide salinity range, from the low water line to above the reaches of the tide, and are usually not submerged. They are detritus-aufwuchs feeders (Odum and Smalley, 1959), and are constantly active during the warmer months, but only on the warmer days of winter. When such marsh grasses as Spartina alterniflora become abundant in spring and summer, L. irrorata are found climbing on them. Information on reproduction in this abundant and conspicuous species is absent from the literature. Odum and Smalley (1959) mentioned recruitment in populations of L. irrorata in a paper dealing with energy flow in a tidal marsh; the methods used and other pertinent information are given only in Smalley's

Ph.D. thesis (1960). Woodard (1942a, b) investigated the development and behavior of the apyrene sperm of L. irrorata. Two unpublished studies have dealt with early development. Patsy Ann Smith studied larval development in relation to salinity at the VIMS Wachapreague laboratory, and Jeanette Whipple Struhsaker conducted laboratory investiagations of spawning and early development at the Duke University Marine Laboratory at Beaufort, North Carolina (personal communications).

The objectives of the present study were to: 1) ascertain the time of occurrence and the duration of the breeding season in Littorina irrorata in the Chesapeake Bay area of Virginia; 2) identify environmental factors which might influence reproductive activity; 3) confirm the type of reproductive products liberated; 4) determine if lunar periodicity of release existed; 5) observe and record patterns of behavior associated with reproduction.

CONDITION OF GONADS

Materials and Methods

Specimens examined for maturity of gonads were obtained from Sarah Creek (Fig. 1). Random samplings of at least 50 snails were made at biweekly intervals from 15 March to 18 September 1967, and from 15 April to 20 June 1968. Smaller, more casual collections were made between the biweekly intervals, and from October 1967 to April 1968. Shells were removed from specimens by cracking them with pliers and severing the columellar muscle attachment to the shell. A visible testicular duct in males, and an enlarged capsule gland in females (Figs. 2a and 2b), were interpreted as indicating maturity of the gonads.

In addition to macroscopic examination of the genital system, smears of freshly excised visceral tissue from both sexes were made regularly and examined microscopically for developing gametes. A number of males and females were fixed in AFA during the spring of 1968. Blocks of abdominal tissue were then sectioned and stained in hematoxylin and eosin. The slides were examined microscopically to determine the degree of gonad development.

Difference in degree of gonad development at two-week intervals during May and June of both years was tested for significance by X^2 (Snedecor, 1956). Correlation of gonad maturation with rising spring temperatures and with increasing photoperiod was attempted. Air temperatures for the two years were obtained from the hygrothermograph

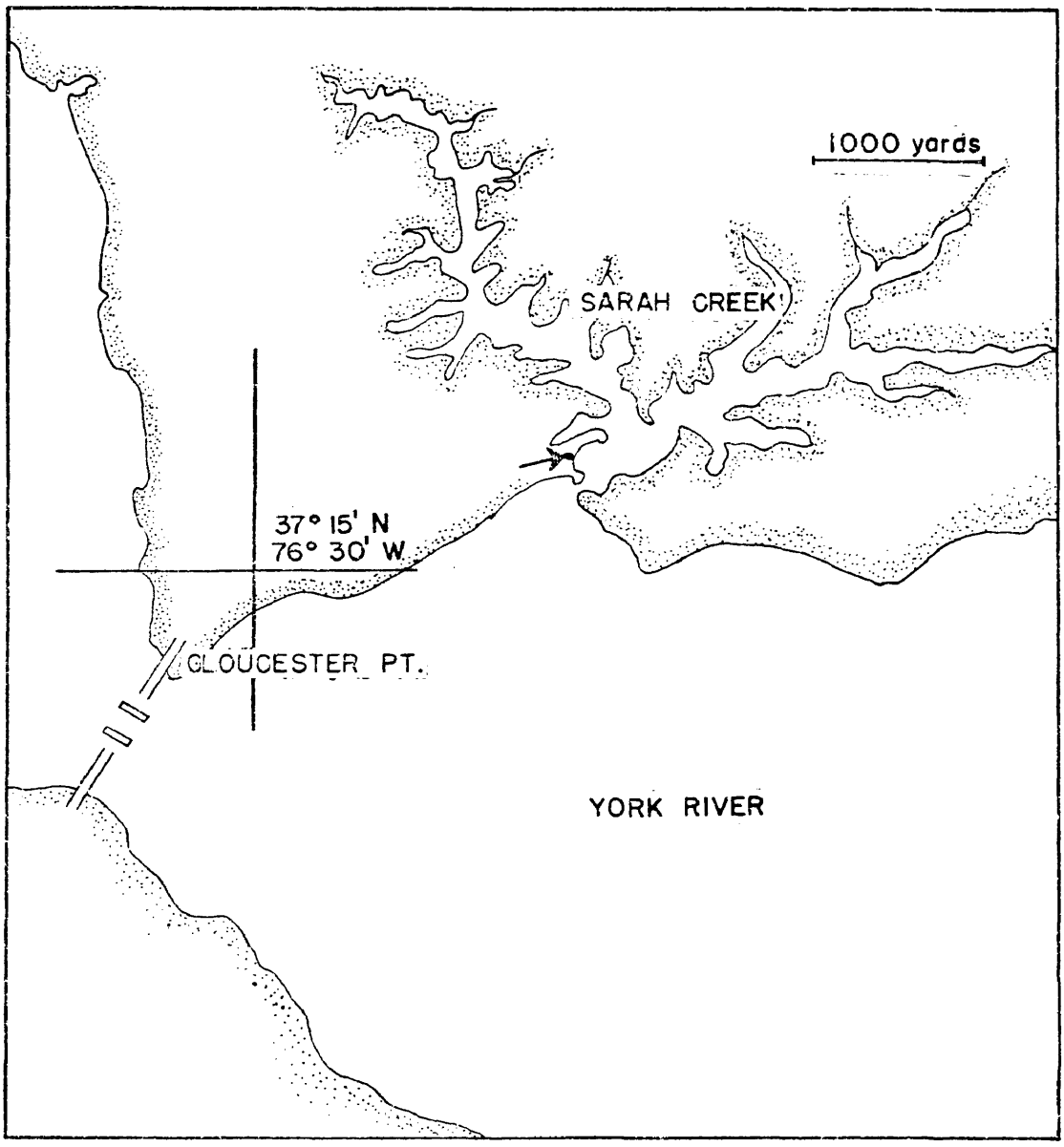


Figure 1. Major site of collections and observation of adult Littorina irrorata (arrow); plankton tows were made in the water near the site.

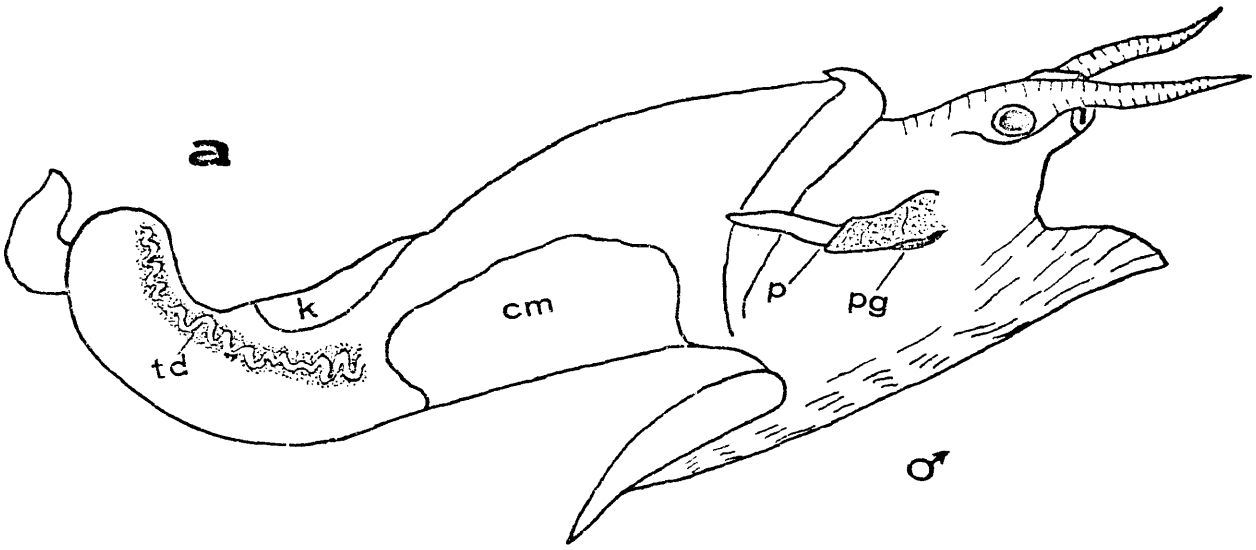


Figure 2a. Sexually mature L. irrorata male removed from shell. Overall length, 25 mm; cm: columellar muscle; k: kidney; p: penis; pg: penial gland; td: testicular duct. Testicular duct visible only when filled with spermatic fluid, during breeding season. X6.

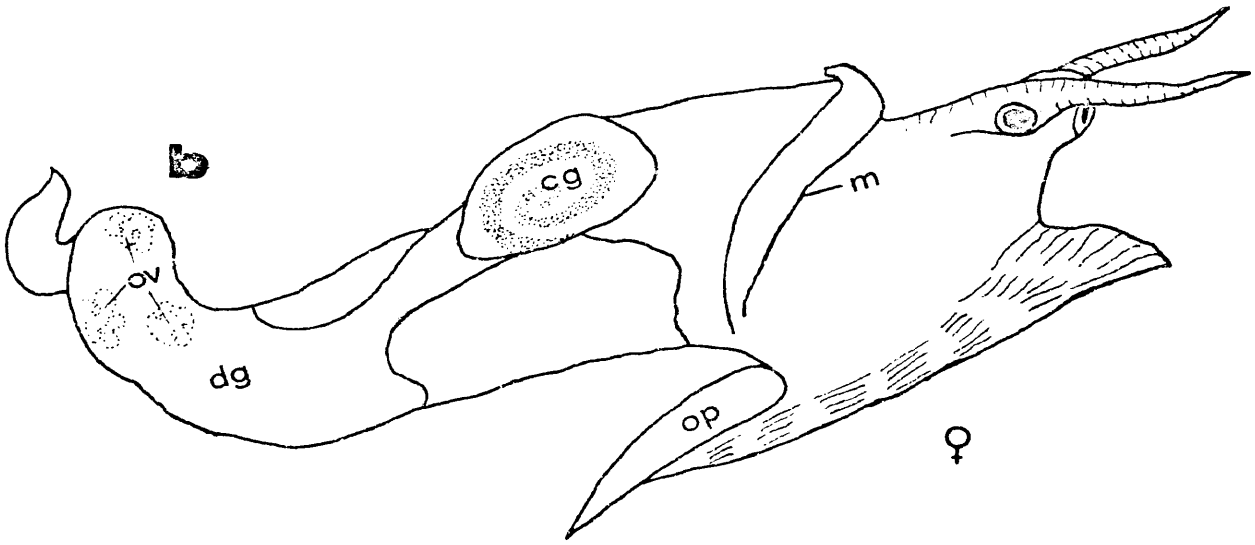


Figure 2b. Sexually mature L. irrorata female removed from shell. cg: capsule gland; dg: digestive gland; m: mantle edge; op: operculum; ov: ovaries. Ovaries seldom easily visible; capsule gland encapsulates zygotes, is prominent only in breeding season. X6.

at Virginia Institute of Marine Science (VIMS), approximately one mile from Sarah Creek. The means of daily highs, lows, and means from corresponding months were compared by graphing the standard errors of the means, and significant differences were confirmed by a t-test (Snedecor, 1956). Water temperature records from the VIMS recording thermometer were incomplete for April and May 1968, but comparisons between the two years were attempted. Daily photoperiods were calculated from the Old Farmer's Almanac (Thomas, 1968).

Results

The first sexually mature individuals were found 24 May 1967. A survey of 76 randomly-selected snails on that date showed that 42.5% of the 40 females and 44.4% of the 36 males examined were mature. A similar survey two weeks later showed a significant increase in sexual maturity: 81.1% of 69 females and 65.5% of 35 males possessed mature gonads. By 21 June 1967 no individuals with immature gonads could be found. A survey taken 18 September 1967 showed gonad maturity waning: 32.8% of 67 females and 12.2% of 41 males still possessed mature gonads.

Gonad maturation commenced earlier in 1968. No mature individuals were found 2 May, though several were found during the two weeks following. By 16 May 78.4% of 23 females and 76.6% of 43 males were sexually mature. On 24 May 100% of 77 females and 73 males examined had mature gonads. The data for both years is presented in Fig. 3. A X^2 test at 95% confidence interval confirmed that the percentage of sexually mature snails found 24 May, 1968 compared with the survey of 24 May 1967, was significantly greater.

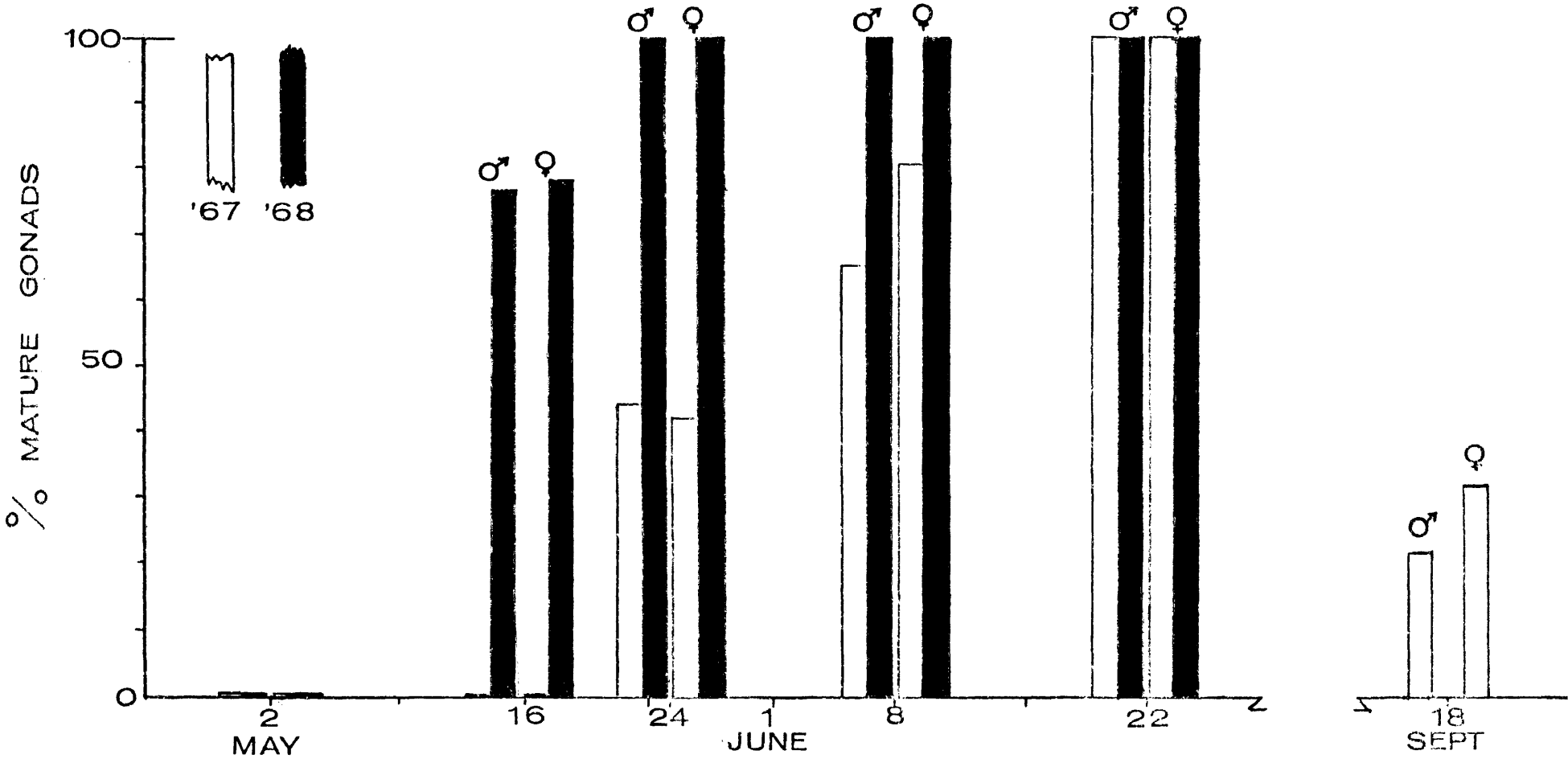


Figure 3. Percentages by sex of individuals with mature gonads in May-June 1967 and 1968; gonad maturity 18 September 1967. Determined from macroscopic examination; see text for numbers of animals used.

A transverse section through the abdomen of a female fixed 2 May 1968, showed no signs of developing eggs. A similar section of a female fixed 24 May showed maturing oöcytes (Fig. 4). No sperm were seen in the testis of a male fixed 2 May 1968 (Fig. 5a), but mature eupyrene sperm and their accompanying apyrene cells were seen, along with developing germ cells, in a section of visceral sac from a male fixed 16 May (Figs. 5b, c, d). These results were as expected from the surveys taken on these dates and were further corroborated by the male and female gametes found in live smears. A fully developed spermatozeugma (apyrene sperm with eupyrene sperm attached; Woodard, 1942a, b) from a testicular duct is depicted in Fig. 6.

Increasing air temperature was regarded as more likely to stimulate gonad maturation in the spring than rising water temperature, because L. irrorata was very seldom submerged, and because air temperature increase occurs earlier and more rapidly than rises in water temperature. The daily high and low air temperatures for March through June 1967 and 1968, are given in Fig. 7. The most discernible trend toward warmer temperatures in 1968, when the population of L. irrorata matured sooner, is seen in the four-week period from 8 May to 4 June. Between those dates in 1967, the temperature fell to 10C or below six times; in 1968, only once. The means, ranges, and $S_{\bar{X}}t_{05}$ (standard errors of the means) of daily highs, lows, and means for this period in both years is given in Fig. 8. The difference between the means of daily highs was not significant, but monthly means of both mean and low temperatures were significantly higher in 1968. These results were confirmed by t-tests at the 95% confidence interval (Snedecor, 1956). Similar t-tests for April in the two years

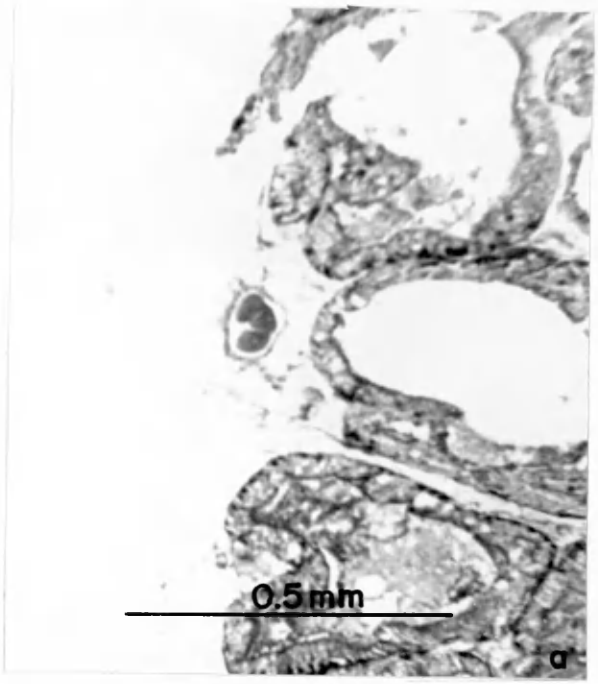
Figure 4. Photomicrographs of maturing primary oöcytes in ovaries of female L. irrorata fixed 24 May 1968. Hematoxylin and eosin stain.

a) Ovarian tubule containing oöcytes; surrounding digestive tissue. 82X.

b) Oöcytes pictured in 4a under oil immersion. Note large nucleolus (n), typical of primary oöcytes. 820X.

c) Primary oöcytes. Meiotic divisions will not occur until after sperm penetration. 360X.

d) Primary oöcyte under oil immersion; n: nucleolus. 820X.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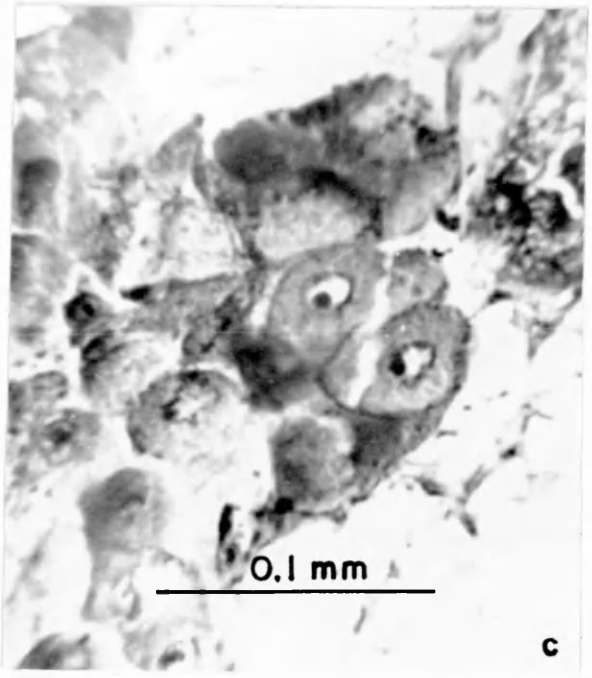
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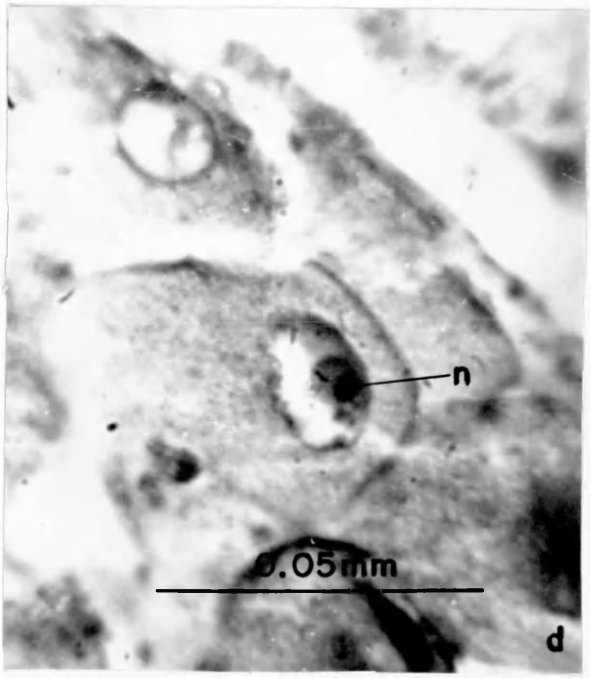
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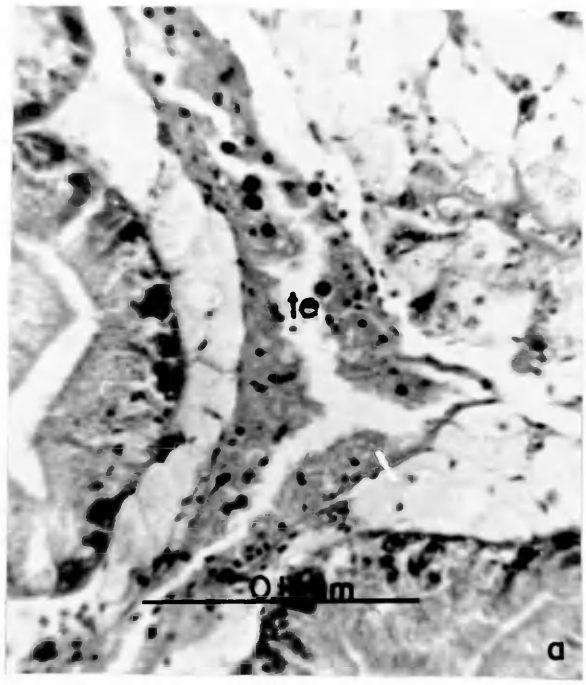
Figure 5. Photomicrographs of testes and developing sperm from section of male L. irrorata digestive glands. Hematoxylin and eosin stain.

a) Testis lacking mature gametes (te) from male fixed 2 May 1968. 360X.

b) Fully developed testis from male fixed 16 May 1968. Central portion (in rectangle) shown in 5c; dg: digestive gland; te: testis. 82X.

c) Detail of 5b. Dark areas are nuclei of spermatocytes and spermatids; as: apyrene sperm (see Fig. 6). 360X.

d) Testis of male fixed 16 May 1968, under oil immersion as: apyrene sperm; ds:developing spermatocytes; sh: sperm heads; st: sperm tails. 820X.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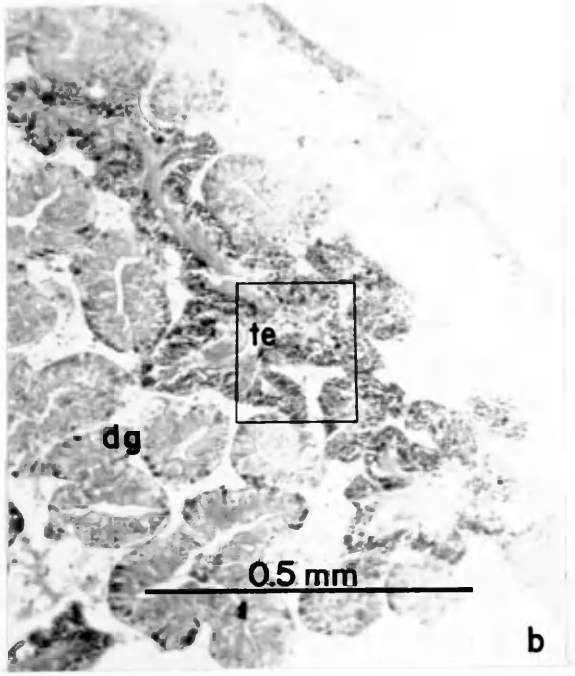
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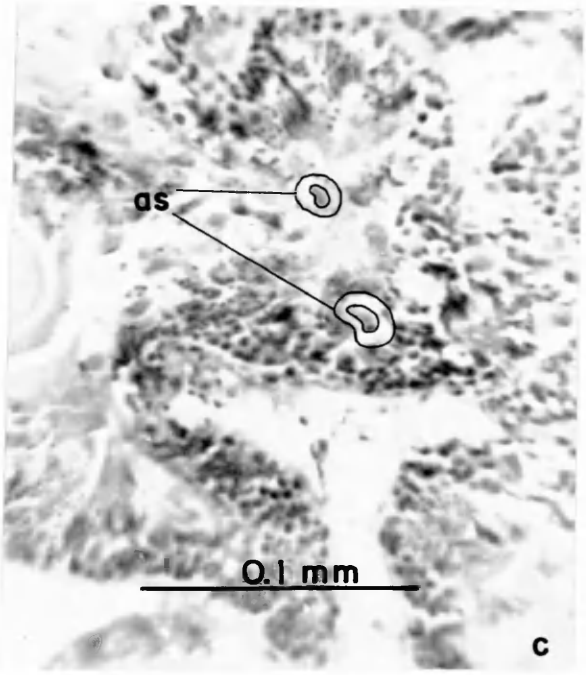
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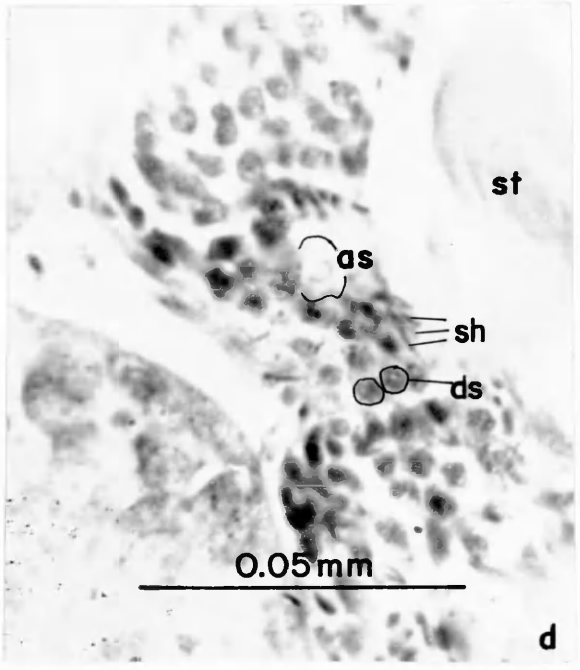
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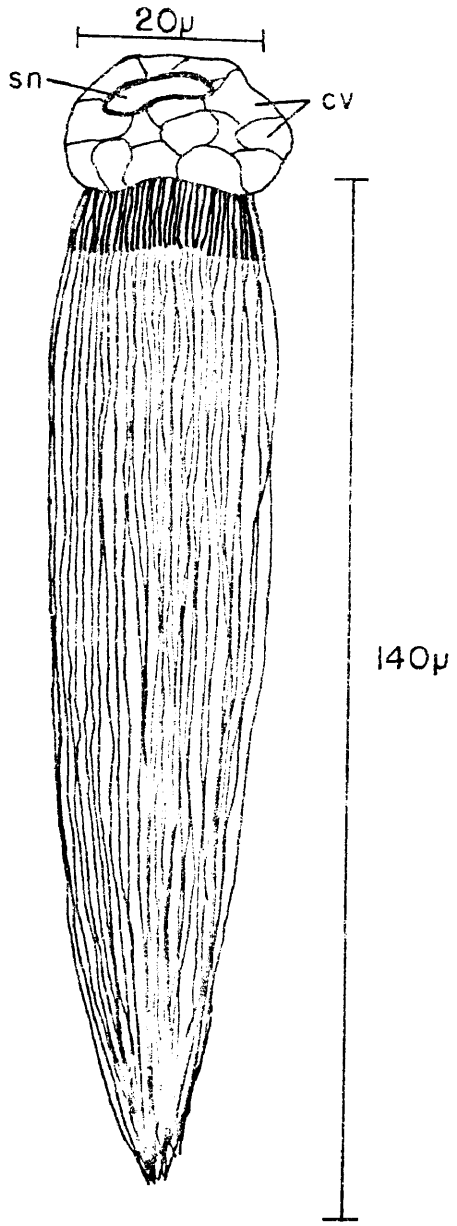


Figure 6. Apyrene sperm of L. irrorata with eupyrene sperm attached; sn: fused sperm nuclei; cv: chromatic vesicles, remnants of the cell nucleus. The dark band just below the apyrene cell is formed by sperm heads. See text for summary of apyrene sperm development. Drawn from life.

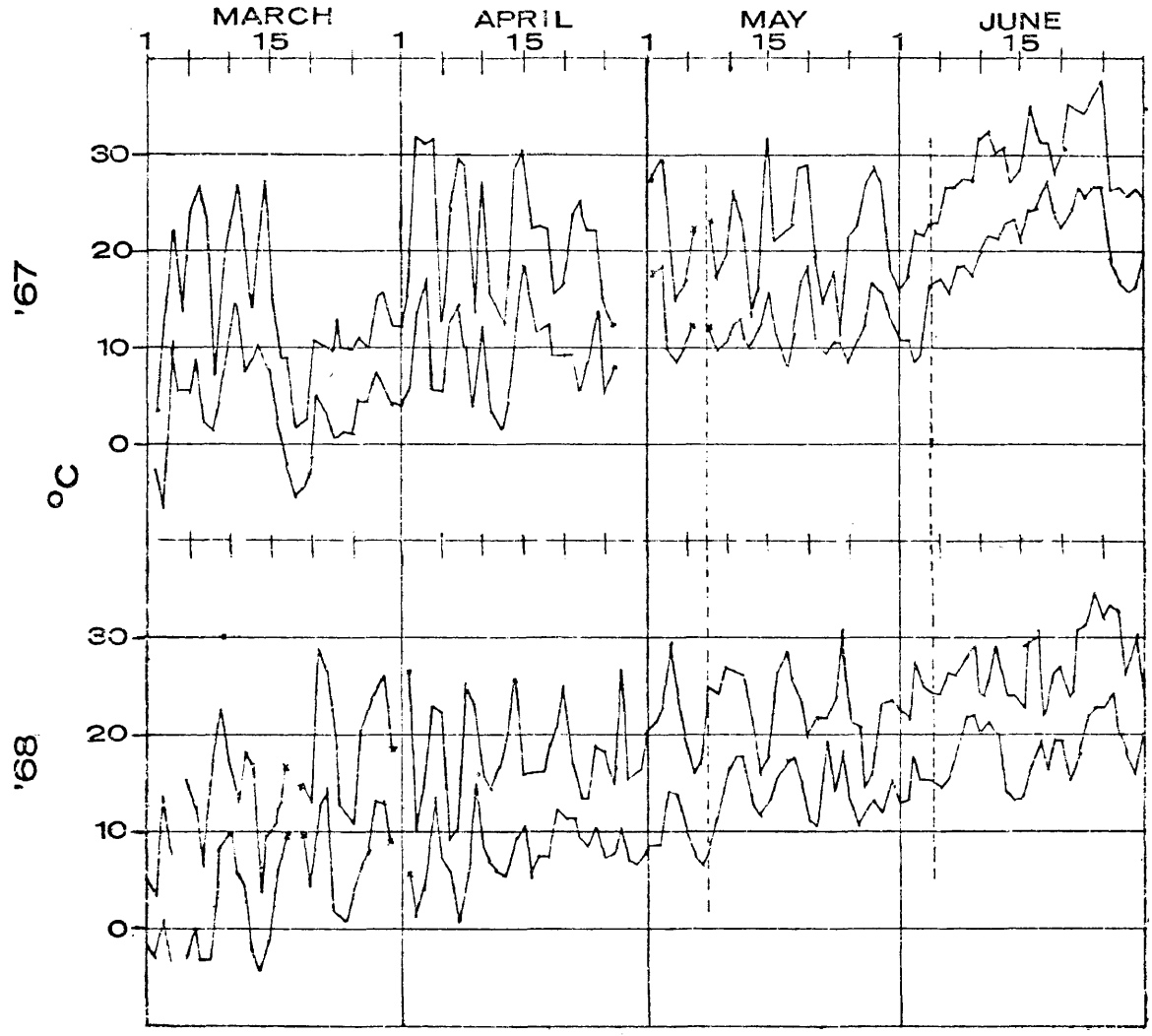


Figure 7. Daily high and low air temperatures for March through June, 1967 and 1968. Breaks in lines indicate lack of data due to equipment failure. The temperatures between the dashed lines are treated statistically in Fig. 8.

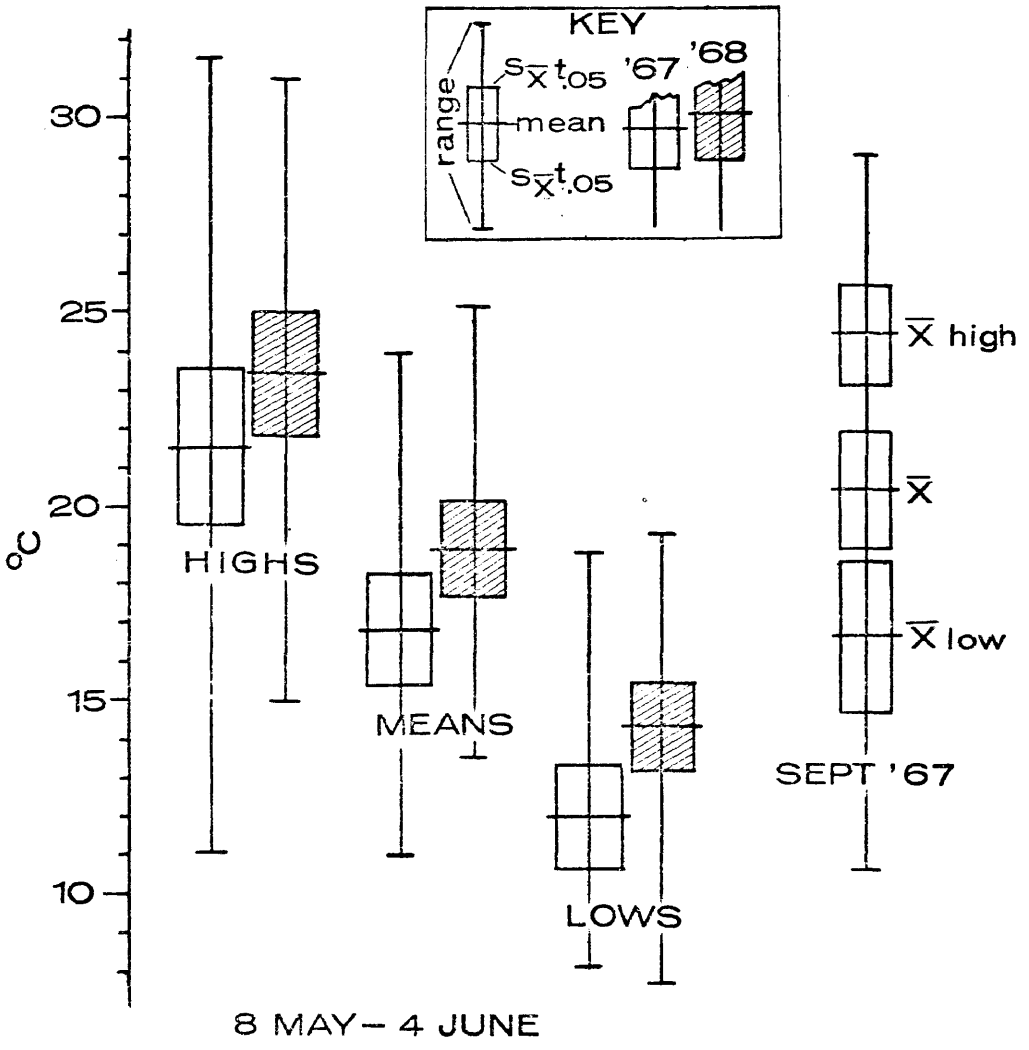


Figure 8. Comparison of standard errors of the means ($S_{\bar{x}}t_{05}$) of daily highs, lows, and means for period 8 May -4 June, 1967 and 1968. Similar data for September 1967 also presented. Non-overlap of $S_{\bar{x}}t_{05}$ of one year group with mean of comparable group from other year indicates significant differences. See text for details.

revealed a reversed situation: highs, means, and lows for April 1967 were all significantly warmer than for April 1968.

The means of daily highs, lows, and means for September 1967, the month in which gonad maturity began to wane, were significantly higher than for May of that year, when gonad maturation began; they did not differ significantly from the data for May 1968.

Available data indicated monthly highs, lows, and means of water temperature from March through June seldom differed by more than 1C between the two years for any particular period. Incomplete data made further comparison impossible.

Photoperiod, calculated as time from sunrise to sunset, was 14 hours, 14 minutes for 10 May, the earliest date on which any mature snails were found in either of the two years. The photoperiod for 18 June, the latest date on which any immature individuals were found, was 14 hours, 52 minutes. A photoperiod difference of 38 minutes thus existed over the maturation period combined from the two spring seasons.

Surveys in mid-July revealed that both the smallest and largest snails included in samples were reproductively mature; apparently all snails past the juvenile stage are members of the reproductive population, and senescence does not impair reproductive potential.

Some of the genital organs of L. irrorata regressed in winter; the capsule gland of females was much reduced in size, and the male testicular duct was not visible. The penis, however, showed neither the enlargement concomitant with gonad maturity, nor the post-reproductive atrophy mentioned for other species (Linke, 1933b).

Discussion

Littorina irrorata is unquestionably a summer breeder, but the factors influencing gonad maturation are still in doubt. Indeed, the mechanism of gonad maturation in the genus *Littorina* is not clearly understood. Rohlack (1959) demonstrated an estrogen-like hormone in fertile females of *L. littorea*, but could demonstrate no sex hormone in sexually mature males. The influence of temperature on genital maturation and degeneration has been shown in *L. littorea*, which breeds in the spring after a gonad buildup initiated in October and continuing through the winter. Linke (1934) found degeneration of the testes and penes of males that were held at summer temperatures (14-26C) for five to six weeks during the spring breeding season; he induced the same result by starving other animals. Rohlack (1959) found that in 80% of *L. littorea* held at winter temperatures from June through August, the gonad maturity normally found only through May had been retained. Gonad activity was advanced in both male and female *L. littorea* held at 1-5C during September and October; 40% of the males and a smaller, unspecified percentage of females possessed mature gonads, while in the field maturation was not detectable (Rohlack, 1959). In both experiments with reduced temperature, Rohlack maintained natural environmental photoperiods by use of glass-doored refrigerators.

Although the yearly temperature cycle is apparently linked to the reproductive cycle in *Littorina*, effects are far from uniform: rising temperatures are linked with initiation of reproduction in some species, as in *L. irrorata*, while they accompany lowered fertility in others, e.g., *L. neritoides* (Palant and Fishelson, 1968; Lysaght, 1941). In the present study, decreased gonad maturity was observed in September

1967, when temperatures were significantly warmer than in May, when it commenced. Therefore, the minimum temperatures required for maturation in the spring were not effective in preventing gonad degeneration in the fall. Other factors which may account for degeneration of gonads while temperatures are still favorable must be looked for; shortening day length and drop in temperatures from those of the preceding month may be involved. If temperature changes mediate the reproductive cycle, they are probably far less in magnitude than those indicating statistically significant difference. In a case such as this, statistical confirmation of the animals' response to differing conditions would be more valid than confirming a difference between the conditions themselves.

Photoperiod has not been suggested as a factor influencing the reproductive cycle of Littorina, even in the most recent papers (Palant and Fishelson, 1968; Struhsaker, 1966; Rohlack, 1959); nor is it mentioned in the most recent reviews of molluscan and prosobranch physiology (Hyman, 1967; Wilbur and Yonge, 1966; Fretter and Graham, 1962). Rohlack's experiments in which she lowered environmental temperatures but permitted normal photoperiod indicated that temperature alone has some effect as a mediating factor, though response in experimental animals was never 100%. If lengthening photoperiod does influence gonad growth in L. irrorata, the earlier attainment of maturity by 100% of the population in 1968 (24 May as compared with 21 June a year earlier) could be explained by the concurrent influence of warmer temperatures. A plant will bloom, once light period is sufficient, only as rapidly as climatic conditions will permit (Bissonette, 1936). The warmer temperatures and earlier maturation in May 1968 were preceded by a month that was significantly colder than the same period in 1967. It is

likely that the critical effect of temperature does not express itself until sometime later than April, and minimum required photoperiod may be the reason. On the other hand, rate of temperature change may be as much an initiating factor as photoperiod or final temperature reached.

It may be that some environmental factors act indirectly in influencing gonad development. Photoperiod and temperature certainly affect the food supply of these grazing animals. Since Linke (1934) found genital degeneration in starved L. littorea, a reverse effect is possible and abundant food could trigger a physiological response leading to reproductive activity.

Whatever environmental factors may influence the reproductive cycle in Littorina, their mediation cannot be understood without further physiological work, as a male hormone has yet to be demonstrated, and the stimulus for the seasonal secretion of the female hormone is unknown (Rohlack, 1959). In the absence of knowledge of a male hormone in Littorina, the physiological implications of the retention of a fully-developed penis throughout the year in L. irrorata are uncertain. Degeneration is almost complete in L. littorea, but insignificant in L. saxatilis (Linke, 1933b).

Apyrene sperm are found in almost all prosobranch gastropods. Littorina is one of the few genera in which they are sometimes lacking, though they are present in L. irrorata (Woodard, 1942b). These auxiliary cells develop in the testes from cells indistinguishable from normal spermatogonia, but they have certain oöcytic properties. Their development involves penetration by several eupyrene sperm followed by sequential mitotic and amitotic divisions of the cell nucleus. The

resultant nuclei fragment into several chromatic vesicles and ultimately disappear. At the stage when several hundred sperm attach their head ends to the cell surface, the fused sperm nucleus represents the only coherent chromatic matter in the cell (Woodard, 1942b). Normal sperm remain attached to the apyrene sperm and are transported to the female genital system in this condition.

Development studies on dioeceous prosobranchs with external fertilization have shown that both polar bodies are extruded only after sperm entry of the egg (Kessel, 1964). Therefore, the maturing germ cells in the ovary only reach the stage of primary oöcyte, and will not undergo reduction divisions until sperm entry. The large nucleoli of the cells in Fig. 4 are characteristic of primary oöcytes, as the nucleolus is not visible during or after meiosis. In L. irrorata, penetration of the oöcyte by a sperm must occur internally, before encapsulation. The site of fertilization is probably the receptaculum seminalis (Fretter and Graham, 1962).

Since all snails surveyed in July were sexually mature, it is evident that no juveniles were encountered in sampling. Juveniles are not found within the habitat of adults; they are found instead among Spartina roots or at the junction of Spartina leaves and stems, and leaves must often be stripped from stems in order to reveal the juveniles (Smalley, 1960). Attainment of sexual maturity requires two years (Smalley, 1960; Struhsaker, personal communication).

RECOVERY OF PLANKTONIC CAPSULES AND LARVAE: LUNAR PERIODICITY IN SPAWNING

Materials and Methods

The first attempts to recover released capsules were made in the laboratory. To this end, samples of 10 snails were brought into the laboratory at least twice weekly from 1 May to 30 August 1967. Females from copulating pairs were taken whenever they were found. These animals were held in glass jars, some jars being partially filled with York River water, others completely filled. The partially filled jars enforced a high-tide situation, after the method of Kojima (1958a, b; 1959). All jars were covered with a transparent polyethylene film which prevented escape of the animals and evaporation of water, but permitted diffusion of gases and passage of light. Water was changed twice weekly; salinity varied from 17.9 to 22 °/oo.

As soon as the first capsules had been obtained in the laboratory, a plankton sampling program was begun. A small net of monofilament polymer, #2 mesh (openings of 315μ on a side), was towed daily through the inshore water at Sarah Creek (Fig. 1) from 21 July to 5 October 1967. The tows were made in the afternoon each day, so all stages of the tide were sampled sequentially. Additional tows were taken at high water during each full and new moon.

Plankton sampling was begun in 1968 before any evidence of gonad maturity had been found. Daily tows were made at high tide from

2 May to 24 June 1968, at the same site used in 1967, with the exception of 10 June, when a tow was made at Wachapreague, Virginia. Weekly tows were made at high tide from 24 June to 17 August 1968. Some tows were also made at low and intermediate stages of the tide, though not regularly. The suggestion was made that some of the discoid capsules (Figs. 9a, b, c), 425μ in diameter, could have slipped diagonally through the #2 net mesh, so a #20 mesh net (openings 40μ on a side) was used in 1968. It was once towed in tandem with the #2 net for purposes of comparing capsule retention.

Development of captured zygotes was observed in water of 31.8 °/oo salinity, to determine approximate time required for attainment of various stages.

Moon phases and tidal stages were obtained from the Tide Tables of the U.S. Coast and Geodetic Survey (1967 and 1968).

Results

The first laboratory spawning occurred 21 July 1967, coincident with a full moon, in a jar containing 10 snails that had been brought in the day before. The jar was one that had been only partially filled with river water. All of the approximately 50 encapsulated embryos had developed to the trochophore stage when first observed. A second spawning occurred the next night. Although the laboratory procedure for obtaining oöthecae was continued, no more were seen through termination of the procedure at the end of August. No oöthecae were recovered from the plankton in 1967, despite daily tows from 21 July until October. Some veligers were recovered, which were identified for me as L. irrorata by Dr. Jeanette Whipple Struhsaker of

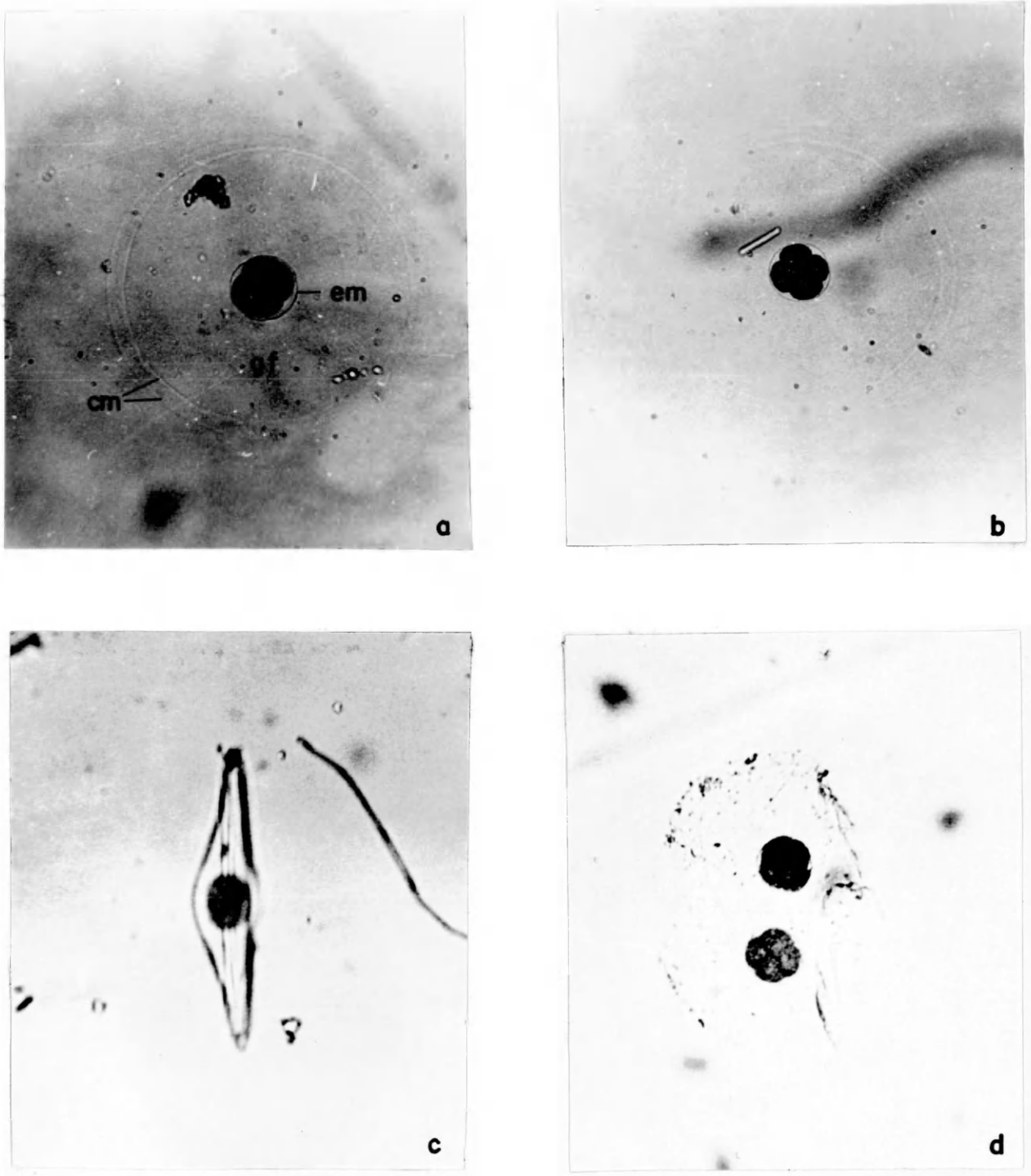
Figure 9. Encapsulated zygotes of L. irrorata, preserved in 10% buffered formalin. 115X.

a) Zygote after first division; em: embryo membrane, diameter 90μ; cm: capsule membrane, 20μ in thickness; gf: gelatinous fluid between membranes. Outside diameter of capsule: 425μ.

b) Zygote in four-celled stage.

c) Capsule in side view, showing discoid nature; thickness at center: 130μ.

d) Capsule containing two embryos. This phenomenon was observed only once. The crenations of the capsule edge were present when the specimen was taken.



0.5 mm

the University of Hawaii. She had raised zygotes of L. irrorata to adult snails. The veligers were taken at all stages of the tide, but more frequently at high water, when as many as 12 were taken.

In 1968, plankton tows from 2 May to 9 June yielded only one oötheca, which was crenated and contained a darkened embryo, indicating it was dead. On 10 June coincident with a full moon, thousands of capsules were taken at high tide at Wachapreague, Virginia. When some snails collected at low tide earlier that day were covered with water, they spawned immediately. All these embryos were in the one-celled stage when collected. Within an hour, some had divided twice to the four-celled stage (Fig. 9b) in water of 31.8 °/oo salinity. Some 16-celled stages appeared when the embryos were three hours old, and within 12 hours, all were trochophores.

Large numbers of capsules were taken at Sarah Creek 11 June, and at high tide every succeeding day until new moon, when sampling was reduced to a weekly basis. Large numbers were taken in every weekly plankton tow made at high tide until these were discontinued in mid-August. Embryos had never developed past the four-celled stage at the time of high-tide collection. Oöthecae were never found inshore at low tide, but a few were taken in two meters of water when the tide was out. The embryos had divided into 16 cells. Only once in the observation of many thousands of oothecae was a capsule seen to contain two embryos (Fig. 9d).

A simultaneous towing of the #2 and #20 mesh nets on 18 June 1968 revealed no visible difference in the quantity of eggs retained. Encapsulated zygotes were found abundantly in both nets.

The oöthecae of L. irrorata are discoid in shape, with the embryo turning freely within. The diameter of the outer capsule membrane was 425μ by my measurements, slightly larger than the dimensions given by Smith and by Struhsaker in their unpublished studies. This outer membrane is about 20μ thick; the space between it and the spherical egg membrane is filled with a gelatinous material. The egg membrane is 90μ in diameter (Figs. 9a, b). The egg capsule is about 130μ thick at the center, and appears ellipsoid in side view (Fig. 9c).

Discussion

The techniques used for collecting capsules both in the field and in the laboratory were similar for 1967 and 1968; the great success in the latter year and the all but complete failure in the former indicates that there may have been a paucity of capsules released in 1967. The release of capsules in the laboratory that year and the field collection of veligers indicates that some spawning did occur, but scarcity seems to have been the rule. This scarcity cannot be attributed to lack of gonad maturity within the population, as it was shown to be 100% by 21 June. It was shown by the results of 1968 that no capsules should have been expected in plankton tows made at low tide. Still, not a single oötheca was taken even on spring highs in 1967, whereas results for those periods in 1968 were dramatically positive. The possibility exists that a great deal of field spawning took place in 1967 before plankton sampling was begun. If so, laboratory animals did not reflect this, whereas in 1968 laboratory animals closely reflected occurrences in the field. Also, this would indicate an earlier spawning in a year when gonad maturity was attained later,

an unlikely situation. Odum and Smalley (1959) suggest that adult populations of L. irrorata receive significant replenishment only once in several years, when an unusually heavy set of larvae occurs. If this is true, the heavier set might well be directly related to more larvae from a greater number of capsules released. Such a phenomenon, though unexplained, may be tied to the great abundance of capsules taken in 1968 compared with the extreme paucity in 1967.

Plankton samples from 1968 revealed that L. irrorata release oöthecae during all phases of the moon, but only at high tide. Some lunar periodicity is indicated, since the only capsules obtained in 1967 were released on the full moon, and the first significant release in 1968 was also coincident with a full moon. Dr. Struhsaker found that snails brought into the laboratory less than 24 hours before, spawned at the time of both high high and low high water in the field during spring tides at Beaufort, N.C., but only at high water during neaps in 1965 (personal communication). All available information points to a slight periodicity in spawning, and great yearly variation in the abundance of spawn.

Because populations of L. irrorata were not submerged at low tide, release of planktonic capsules would have been impossible at such times. Capsules did not remain inshore after release, since the only tows which yielded any at low water were those taken at two meters depth. The 16-celled condition of those indicated that they had been released at least three hours before, and also that they tended to sink during development. Their downward drift would naturally carry them away from shore on the ebbing tide.

Unpublished work done in 1965 by Patsy Ann Smith, formerly of Great Bridge High School, revealed that zygotes of L. irrorata developed fastest at 30 °/oo, while 20 °/oo was adequate for development but 10 °/oo was not. Development to the 16-celled stage would then have taken longer at the salinity in Sarah's Creek (17-22 °/oo) than the three hours development time observed at 31 °/oo. Any oöthecae found at that stage offshore were therefore probably released on the preceding high tide.

The capsules of L. irrorata resemble those of most other oviparous Littorina in size and shape. Smalley (1960) was obviously in error when he reported that the capsules of L. irrorata were spherical; apparently he did not observe any in side view. Since no congeners are found in the habitat of L. irrorata, the problem of assigning capsule types to different species did not arise. The capsules of some other species normally contain more than one zygote (Lebour, 1937; Kojima, 1958a), but the single observance of two embryos within a capsule of L. irrorata indicates that this was an anomaly.

FIELD OBSERVATIONS OF REPRODUCTIVE BEHAVIOR

Materials and Methods

Field observations were made near the collecting site (Fig. 1). In observing copulating pairs, their position was noted, and date, time of day, and phase of tidal cycle were recorded. Some copulating pairs were pulled apart to ascertain the length of the penis during copulation. The sex of the inferior partner in such cases was also checked, to see if males ever attempted copulation with other males as described in L. planaxis (Gibson, 1964) and L. littorea, L. obtusata, and L. saxatilis (Linke, 1933a). Forty-five copulating pairs were marked with nail polish 25 June 1967; both members of each pair received a distinguishing mark on their shells. Subsequent pairing behavior was then noted once daily, on an ebbing tide, for four days.

The amount of submergence of the habitat of the L. irrorata population was observed through a complete tidal cycle 8-9 June 1967. The rise and fall of the tide was recorded hourly for 25 hours. The movements of the snails in a meter-square plot (Fig. 10) were also noted for this time period. The percentage of snails which had moved to the back of the plot and climbed onto the Spartina was plotted against the tide level.

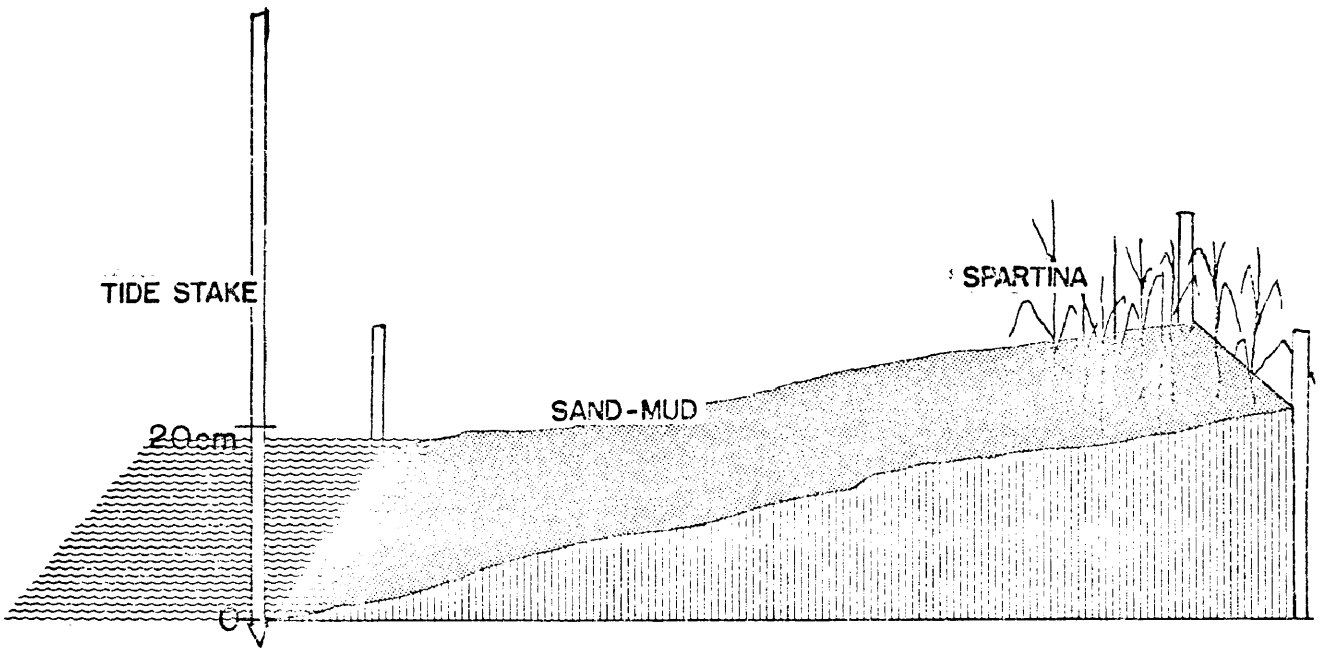


Figure 10. Diagram of meter-square plot observed over a complete tidal cycle (25 hours) 8-9 June 1967. Plot was located at site in Fig. 1. Plot was completely exposed at LLW and HLW; completely submerged at both LHW and HHW. Some results of observations here are given in Fig. 12.

Results

Copulation was most often observed on the ebb tide, seldom when the animals were submerged, and never on vertical surfaces. It was seen to occur every day from the end of May to the end of August, 1967. Copulatory position was the same as described for other species (Gibson, 1964; Linke, 1933b): while the female remains with her foot attached to the substrate, the male climbs onto her shell and migrates to the right side, so that his head is pointed in the same direction as hers and the lip of the right side of his shell overhangs that of hers (Fig. 11). In this position, the penis is inserted. The organ becomes engorged with blood and begins to lengthen. The proximal portion is inserted first, with the tip trailing in after. The unpigmented distal portion undergoes great elongation and eventually contacts the female genital pore in the rear of the mantle cavity; sperm are then transferred. When copulating pairs were abruptly pulled apart, the penis of the male was often as long as 25 mm, as compared to about 5 mm in the detumescent state. Mucous secretions furnished by the leaflike adhesive gland at the base of the penis (Fig. 2a) aided in holding the penis in place during copulation.

In some disrupted pairs, the snail in the female position was found to be a male; this indicates that, as in other species, males cannot distinguish the sex of other snails without exploration of the mantle cavity by the penis.

Both males and females from pairs marked with nail polish were found copulating with other individuals on subsequent days; occurrence may well have been several times more frequent than that observed. Great numbers of L. irrorata were seen seaward of the Spartina

Figure 11. Copulatory position in L. irrorata as normally seen from above (top), and as seen in a pair inverted for observation purposes. See text for details. X5.

beds at low water, but as the tide came in, they crawled up the shore and climbed onto the Spartina and other vertical surfaces, thus achieving an elevation often greater than the advancing tide. This phenomenon is quantified in Fig. 12. The number of snails in the meter-square plot (Fig. 10) varied from 65 to 99. The percentage that climbed onto the Spartina rose to 100% as the tide came in; as the plot emerged on the ebbing tide, increasing numbers of snails climbed down from the Spartina and resumed crawling on the sand-mud substrate. Most had been inundated when water level reached 40 cm above the leading edge of the plot, as few of the Spartina stems protruded from the water at that time.

Spring high high water invariably inundated the entire population of L. irrorata; both the rapidity and height of the flood were factors in occluding retreat.

Discussion

Although copulatory behavior itself does not vary from that of other Littorina, adhesion of the penis is accomplished in a slightly different manner, adhesive secretions coming from the gland at the base of the penis instead of from mammaliform glands along its leading edge, as described in other species (Fretter and Graham, 1962; Linke, 1933b).

Since the female supports all the weight of the pair during copulation and only her foot contacts the substrate, copulation on vertical surfaces may be impossible for reasons of increased physical stress and instability. Tactic inhibition of copulation may also occur when the snails are on vertical surfaces; in any case, copulation in such orientation was never observed.

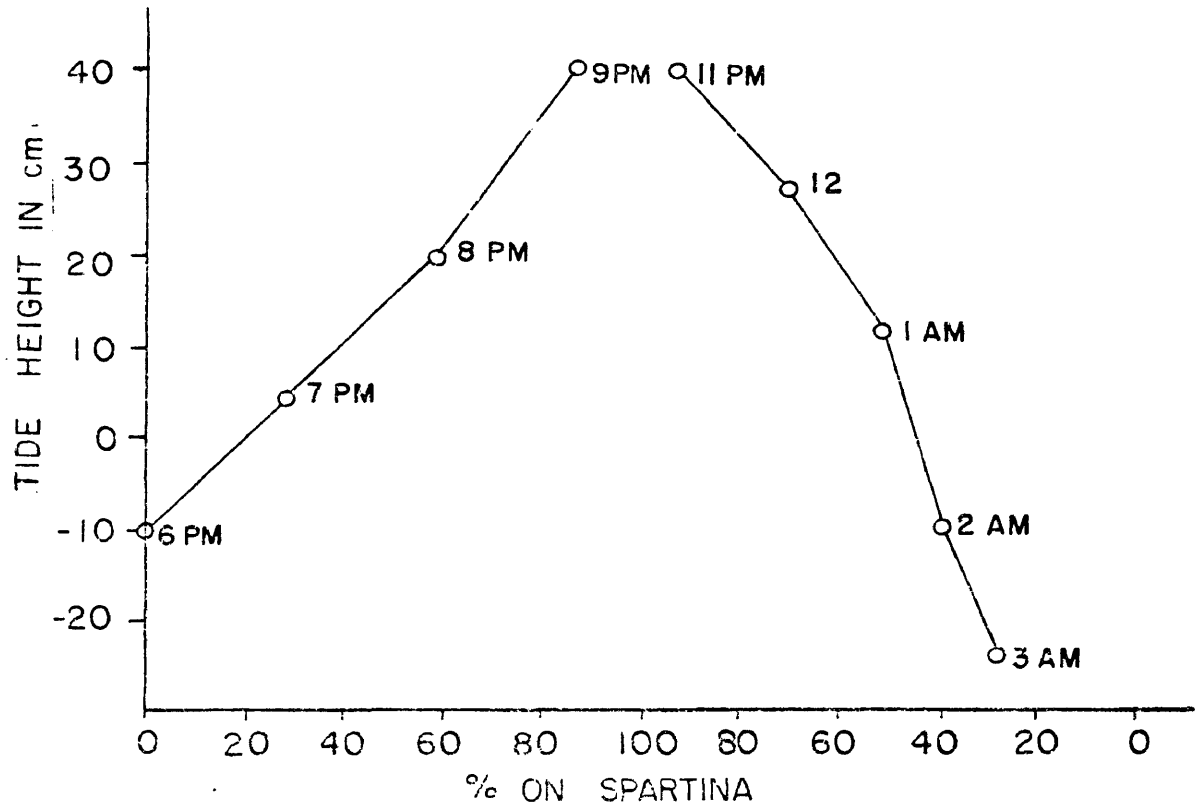


Figure 12. Results of observations of meter-square plot (Fig. 10), 8-9 June 1967. Number of L. irrorata varied from 65 to 99. Snails moved upward to rear of plot and climbed onto Spartina there, thus avoiding inundation until rising tide occluded retreat. As plot was uncovered on ebbing tide, snails left Spartina and grazed on the damp substrate again.

I described homosexual pairing in L. planaxis (Gibson, 1964); Linke (1933b) described it in L. littorea, L. saxatilis, and L. obtusata. Its occurrence is therefore present in congeners from the European and both American coasts, as well as in Hawaiian species (Dr. Jeanette Struhsaker, personal communication). Since sperm is transferred only when males contact the female genital pore, the trait is not selected against except in the sense of time wasted. Indeed, attempted copulation with every other Littorina encountered may be selected for, since the more attempts are made, the more females will be encountered eventually. Homosexual pairing argues against the existence of any female chemo-attractant, except a diffuse one that might enhance general sexual activity in males.

Spring tides are an obvious stimulus to the release of pelagic capsules, since spring high high water occludes retreat and makes the normal submergence-avoidance behavior of L. irrorata ineffectual. During neap high tides, however, retreat to a level above the encroaching tide line is often possible. Since the snails generally do avoid inundation, the release of oöthecae on neap highs must involve a behavior reversal on the part of gravid females. They must allow themselves to be submerged in order to release their floating capsules.

CONCLUSIONS

The sexual cycle in Littorina irrorata is closely associated with their increased general activity in the warmer months of the year. Gonads are degenerate from October to April. The testicular duct of the male is invisible during this time, and the capsule gland of the female is much reduced in size. The penis of the male does not atrophy in winter. Gonad maturation begins in both sexes in May and copulation is prevalent by June. Release of encapsulated zygotes occurs on high tides from at least mid-June to mid-August and appears to be much heavier in some years than in others. A slight lunar periodicity in spawning is demonstrable, but some release occurs throughout the lunar cycle. Capsules sink and drift offshore while developing.

The actual effects of increasing temperature and day length on the sexual maturation of L. irrorata can presently only be inferred from their coincident occurrence. Controlled experiments using elevated temperatures in winter and lowered temperatures in summer, as well as experiments employing artificially shortened and lengthened photoperiod, are obvious next steps in understanding these relationships. The collection of similar data on reproduction from the extremes of the habitat range of this species, and comparison to the present findings from Virginia, would also enhance knowledge of environmental effects on reproduction.

LITERATURE CITED

Bequaert, J. C. 1943. The genus Littorina in the western Atlantic. Johnsonia 1(7):1-27.

Bissonette, T. H. 1936. Sexual photoperiodicity. Quart. Rev. Biol. 2(4):371-386.

Fretter, V., and A. Graham. 1962. British prosobranch molluscs. Ray Society, London. 755p.

Gibson, D. G., III. 1964. Mating behavior in Littorina planaxis Philippi (Castropoda: Prosobranchiata). The Veliger 7(2):135-139.

Hyman, L. H. 1967. Mollusca 1; vol. 6 of the invertebrates. McGraw-Hill, New York.

Kessel, M. M. 1964. Reproduction and larval development of Acmaea testudinalis (Muller). Biol. Bull. 127(2):294-303.

Kojima, Y. 1958a. A new type of the egg capsule of a periwinkle, Littorina squalida Broderip and Sowerby. Bull. Mar. Biol. Sta. Asamushi 9(1):39-41.

Kojima, Y. 1958b. On the breeding of a periwinkle, Littorivaga atkana (Dall). Bull. Mar. Biol. Sta. Asamushi 9(1):35-38. (note: The name Littorivaga has been relegated to a subgenus of Littorina; see Bequaert, 1943.)

Kojima, Y. 1959. The relation between seasonal migrations and spawning of a periwinkle, Littorina brevicula (Philippi). Bull. Mar. Sta. Asamushi 9(4):183-186.

Lebour, M. V. 1935. Breeding of Littorina neritoides. J. Mar. Biol. Ass. U.K. 20(2):373-378.

Lebour, M. V. 1937. The eggs and larvae of the British prosobranchs with special reference to those living in the plankton. J. Mar. Biol. Ass. U.K. 22(1):105-166.

Lenderking, R. E. 1954. Some recent observations on the biology of Littorina angulifera Lam. of Biscayne and Virginia Keys, Florida. Bull. Mar. Sci. Gulf Caribbean 3(4):273-296.

Linke, O. 1933a. Beiträge zur Sexualbiologie der Littorinen. Zeitschr. Morphol. u. Ökol. der Tiere 28:170-177.

Linke, O. 1933b. Morphologie und Physiologie des Genital-apparates der Nordseelittorinen. Wiss. Meeresunters. Abt. Helgoland 19: 3-52.

Linke, O. 1934. Über die Beziehungen zwischen Keimdruse und Soma bei Prosobranchiern. Verh. Dtsch. Zool. Ges. 36:164.

Lysaght, A. M. 1941. The biology and trematode parasites of the gastropod Littorina neritoides (L.) on the Plymouth breakwater. J. Mar. Biol. Ass. U.K. 25(1):41-67.

Odum, E. P., and A. E. Smalley. 1959. Comparison of population energy flow of a herbivorous and a deposit-feeding invertebrate in a salt marsh ecosystem. Proc. Nat. Acad. Sci. 45(4):617-622.

Palant, R., and L. Fishelson. 1968. Littorina punctata (Gmelin) and Littorina neritoides (L.), (Mollusca, Gastropoda) from Israel: ecology and annual cycle of genital system. Israel J. Zool. 17:145-160.

Rohlack, S. 1959. Über das vorkommen von Sexualhormonen bei der Meeresschnecke Littorina littorea L. Zeitschr. Vergl. Physiol. 42:164-180.

Rubinchick, M. A. 1961. The biology and reproduction of Littorina littorea of the White Sea. Biol. White Sea, Univ. Moscow 1:215-230. (Abstr.)

Smalley, A. E. (1958) 1960. The role of two invertebrate populations, Littorina irrorata and Orchelimum fidicinium, in the energy flow of a salt marsh ecosystem. Ph.D. Thesis, University of Georgia. Dissertation Abstracts 20(3):2979.

Snedecor, G. W. 1956. Statistical methods. Iowa State University Press, Ames Iowa. 534 p.

Struhsaker, J. W. 1966. Breeding, spawning, spawning periodicity and early development in the Hawaiian Littorina: L. pintado (Wood) L. picta Philippi and L. scabra (Linne). Proc. Malac. Soc. Lond. 37:137-166.

Struhsaker, J. W., and J. D. Costlow, Jr. 1968. Larval development of Littorina picta (Prosobranchia, Mesogastropoda), reared in the laboratory. Proc. Malac. Soc. Lond. 38:153-160.

Thiele, J. 1931. Handbuch der systematischen Weichtierkunde, Vol. 1. 771p. Reprinted in 1963 by A. Asher and Co., Amsterdam, by permission of Gustav Fischer, Stuttgart.

Thomas, E. B. 1968. The old farmer's almanac, 1969. Yankee, Inc., Dublin, New Hampshire. 144 p.

U. S. Coast and Geodetic Survey. 1967. Tide tables for the Atlantic coast of North America. U. S. Government Printing Office, Washington. 289 p.

U. S. Coast and Geodetic Survey. 1968. Tide tables for the Atlantic coast of North America. U. S. Government Printing Office, Washington. 289 p.

Whipple, J. A. 1965. Systematics of the Hawaiian Littorina Ferussac (Mollusca: Gastropoda). The Veliger 7(3):155-165.

Wilbur, K. M., and C. M. Yonge. 1966. Physiology of Mollusca. Academic Press, New York. 2 volumes.

Williams, E. E. 1964. The growth and distribution of Littorina littorea (L.) on a rocky shore in Wales. J. Anim. Ecol. 33(3):413-432.

Woodard, T. M., Jr. 1942a. Behavior of the nurse cells in Littorina irrorata (Say). Biol. Bull. 82(3):461-466.

Woodard, T. M., Jr. 1942b. Development of the nurse cells in Littorina irrorata (Say). Trans. Amer. Microscop. Soc. 61(4):361-372.

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